4/prts

SPECIFICATION

METHOD FOR ASSAYING BIOLOGICAL SAMPLE COMPONENT

FIELD OF THE INVENTION

The present invention relates to assaying means and an assaying method for a specific component in a lipoprotein fraction in the serum by an enzymatic reaction.

BACKGROUND OF THE INVENTION

From old times, a lipoprotein has been fractionated into high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and chylomicron (CM) by an ultracentrifuge operation. Said operation needs skillfulness. After the installation of an ultracentrifuge, a centrifuge is required for over several days. For this reason, many specimens cannot be treated in short time.

Alternatively, a method for mixing the serum with a solution, in which polyethylene glycol or polyanion, such as dextran sulfate and a divalent cation, including magnesium, calcium and etc., are coexisted or a solution in which phosphotungstic acid and a divalent cation are coexisted to precipitate LDL, VLDL and CM, and fractionated only HDL that remains in the supernatant after the centrifuge has become predominant.

The method may be carried out by applying an automatic analyzer that has been widely spread in a field of clinical tests. That is,

a concentration of the cholesterol in the fractionated HDL may be determined by utilizing an enzymatic method for assaying the total cholesterol using an automatic analyzer that has already been established. However, said method also needs a centrifuge operation, which is performed at a low speed, and when mixing the fractionating agent with the serum problems such as artificial quantitation error and confusion of specimens have occurred. In addition, the assay could not be performed simultaneously with the assay of other general biological items. The clinical tests need to be responded quickly and has been demanded to simultaneously assay one test item together with other test items so as to be shorten a time for tests.

On the other hand, there has also been a report that laid importance on the cholesterol value in LDL, which is a clinical risk factor for arteriosclerosis (Standard Value of Total Cholesterol and Reason for Setting thereof; Arteriosclerosis 24(6), 280 (1996)). Currently, the cholesterol value in LDL is obtained from the results for measurements of total cholesterol (T-CHO), neutral fat (TG) and cholesterol in HDL, by introducing an empirical factor in equation. An equation [Friedewald, W.T., et al., Clin. Chem., 18, 499-502 (1972)] is as follows:

Cholesterol value in LDL

= Total cholesterol value - HDL cholesterol value -TG value/5.

Said method can not be established unless all the three items to be assayed are accurately obtained. Further, reportedly, the

TG value of exceeding 400 mg/dL or the cholesterol concentration in LDL of 100 mg/dL or less leads to a failure such that the calculated value does not reflect the cholesterol concentration in LDL (Warnick, G.R., et al., Clin. Chem., 36(1), 15-19 (1990)), (McNamara, J. R., et al., Clin. Chem., 36(1), 36-42 (1990)). Therefore, it has been difficult to detect extraordinary values of cholesterol in LDL which is the object of the assay by this method.

Besides, there has been a method of separating lipoprotein by electrophoresis and measuring the amount of the proteins and a method of assaying the cholesterol in individual lipoprotein by HPLC. The both methods have poor specimen treating capability and need an expensive dedicated apparatus.

Recently, in order to solve the above-mentioned problems in relation to the assay of the cholesterol in HDL, a kit for automatically assaying the cholesterol in HDL has been developed and widely spread. The technologies disclosed in Patent No. 2,600,065, Laid-open Japanese Patent Publication No. Hei 8-201393 and Laid-open Japanese Patent publication No. Hei 8-131195 use a fractionating agent in combination and a metal contained in the fractionating agent as a divalent cation forms insoluble precipitates with a detergent, generally used in the automatic analyzer, and the precipitates accumulate in a waste liquor disposal mechanism in the apparatus and results in causing breakdown.

Further, during the assay reaction, insoluble aggregates are formed to cause turbidity that will affect on the data of assay and not only the turbidity causes measurement errors but also the